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SEPARATION OF THE AMYLOSES IN SOME COMMON STARCHES.

DISSERTATION

Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in the
Faculty of Pure Science of
Columbia University.

BY

HAROLD A. IDDLES, B.S., M.S.

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Acknowledgment

The author wishes to express his thanks to Professor T. Clinton Taylor, at whose suggestion and under whose direction this investigation was undertaken, for his advice and aid which have been of the greatest assistance throughout the work.

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SEPARATION OF THE AMYLOSES IN SOME COMMON STARCHES.

INTRODUCTORY AND HISTORICAL

Starch occurs in nature in the form of granules, each plant species giving rise to granules possessing individual markings and varying in size and shape. The composition of these granules has been of interest to investigators, since at least two varieties of starch may be produced when they become disintegrated, one a "soluble" variety and the other, insoluble.

Nägeli[1] believed that starch granules were formed by intussusception and that the outer layer is the older and the poorer in the elements of water. This outer layer was called starch cellulose while the inner layer was called granulose. On the other hand, Meyer[2] maintained that starch granules are formed in the same manner as crystals, that is, by apposition, and he called the outer portion alpha amylose and the inner portion beta amylose. Maquenne and Roux[3] and other investigators speak of the "soluble" variety as amylose and the insoluble as amylopectin. In the present article, the two terms alpha and beta amylose will be used to designate respectively, the slimy, less "soluble" heavier constituent and the mobile, easily dispersed, "soluble" portion.

In the past, many workers have undertaken the study of these two constituents of the starch granule. Sherman and Baker[4] using potato starch, heated one or two per cent. solutions to 85° C. for one hour. After this heating to disintegrate the granules, the material was centrifuged, and the clear upper layer containing the "soluble" constituent was separated and its concentration determined. The residue after several further washings was separated and the amount estimated. By this process they record a ratio of 80-90% alpha amylose to 10-20% of beta amylose. Tanret [5] successively treated potato starch with water at a temperature of 45°-90°, then allowed sedimentation to take place so that the "soluble" constituent

could be decanted off. In this way the residue or amylopectin was determined to be 70-80% of the total. Maquenne and Roux (loc. cit.) showed that natural starch grains consist of two substances, the principal one (80%) being amylose, which is partially "soluble" in water at 100° and entirely "soluble" when heated with water under pressure.

Mme. Gatin-Gruzewska[6] added to a 3% potato starch paste one-fourth its volume of hot 40% sodium carbonate and treated the mixture with one-third its volume of 95% alcohol. The filamentous precipitate of amylopectin was washed, caused to swell in water, then neutralized and purified by dialysis or decantation. In this way a yield of 40-45% of amylopectin was obtained. Samec[7] states that starch could be broken down to amylose and amylopectin by allowing it to swell in dilute alkali. After slightly acidifying with dilute acid, the amylopectin settled so that the solution of amylose could be separated from the amylopectin. A yield of 56% of the insoluble and 44% of the "soluble" variety was obtained in this way.

Finally, Ling and Nanji[8] have attacked the problem by the use of enzymes. A starch paste heated to 50° was treated with precipitated barley diastase which had been dried by treatment with strong alcohol. In this way the amylose was preferentially attacked and converted completely into maltose in about twelve hours, while the amylopectin was scarcely attacked. By dialysis the maltose was then removed from the amylopectin which was found to be present to the extent of 33%.

In an investigation of the fat associated with corn starch conducted in this laboratory, Taylor and Nelson[9] have shown that the major part of the fatty material present in corn starch cannot be removed by fat solvents before hydrolysis, but that upon subsequent hydrolysis of the starch molecule higher fatty acids were liberated. It was shown also that starch pastes could be broken down partially by the use of enzymes, thereby yielding a residue several times higher in fat content than the original starch. This residue appeared to be similar to the alpha amylose as prepared from the starch granule by other investigators. However, better methods of separation of the

two amyloses were necessary in order to continue the study of the relation of the fat to the starch molecule. With this in view the development of such methods of separation was undertaken and these serve as the basis of this investigation.

Quantitative methods of separation are desirable also, since the products obtained are of importance in theoretical studies. The beta amylose being a much purer form of carbohydrate than starch itself may be used to furnish a homogeneous and reliable substrate for enzyme studies. Further, in the study of the structure of starch by the formation of ethers or esters more homogeneous products and accurate deductions could be obtained undoubtedly by the use of beta amylose as the starting material. The alpha amylose has been considered ordinarily to bear all the non-carbohydrate constituents of the starch granule. Therefore in order to study the role played by these constituents, a ready source of the alpha amylose is desirable. Finally it was of interest to study the general relation of the alpha and beta amyloses in starches of different genera, such as corn, rice and potato, since such starches vary considerably in their properties.

EXPERIMENTAL

Purification of the Starches Used:

Since starch is separated mechanically from the other substances, principally nitrogeneous, which occur with it in the plant, it was first necessary to remove any such impurities. This was accomplished by treating the starch with an alcoholic solution of hydrogen chloride according to the directions given by Taylor and Nelson (loc. cit.). The product obtained in this way was white and still showed the presence of granules when observed under the microscope. A small amount of hydrochloric acid still remained as determined by drying the purified starch and titrating with standard alkali, which showed a content of 0.13% by weight in the case of one sample of corn starch. In order to make sure that this treatment did not remove appreciable quantities of non-extraneous fatty material during the time of treatment, a 500 gm. sample of corn starch was refluxed eight hours each day for six consecutive days and allowed to stand in contact with the purification medium during the intervening time. The sample was then filtered and washed as in an ordinary treatment. By this drastic or prolonged treatment the fatty content as determined by hydrolysis decreased from 0.47% to 0.276% in this particular case.

Gelatinization:

Before purified starch granules can be separated into the two portions alpha and beta amylose, it is first necessary to rupture the outer coating or shell which encases the granule. This rupture or gelatinization may be accomplished by heating a suspension of the starch, by heating under pressure or by chemical agencies such as alkali or solutions of some inorganic salts. Since heating tends to give a very highly hydrated and gelatinous alpha amylose it was decided to use a water solution of ammonium thiocyanate as the active agent to cause swelling and rupture of the granules[10]. During the operation the mass was stirred until homogeneous and studied under the

microscope from time to time. At first the coverings of the granules break and finally become completely disintegrated. When all the granules have undergone this change, 95% alcohol was added to precipitate the gelatinized mass. This was then ground up with successive portions of 95% alcohol, thereby removing practically all the ammonium thiocyanate and dehydrating the product. After drying in a vacuum dessicator the starch was ready for separation studies.

Ultrafiltration Through Permeable Membranes:

The first method employed to effect a separation was the ultrafiltration of the beta amylose away from the suspended alpha amylose by means of permeable collodion filters. This method was selected because suspensions of relatively high concentrations, 8-10% could be used; the filtrate in all cases was perfectly clear, showing a complete separation had taken place, and in most cases the process was rapid, lasting from one to three days.

For the filtration, it was necessary to prepare collodion membranes of varying degrees of permeability which was best accomplished by following the method of Nelson and Morgan[11]. A two per cent. collodion solution was prepared by dissolving pure parlodion* in 75:25 (by weight) alcohol-ether mixture. For each membrane 5 cc. of this solution was run onto a fogged four inch circular glass plate which was floated on a mercury surface. In this way a membrane of uniform thickness throughout could be obtained. When evaporation had taken place to the desired stage, the plate and membrane were immersed in graded alcohol-water mixtures and finally allowed to soak over night in distilled water. During this treatment or by a slight mechanical action the membranes were loosened from the plate and were ready for use. In order to assign a final grade or determine the relative permeability, the ratio of the solvent (that is the weight of the finished membrane less the dry weight) to the dry weight of the membrane was used.

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* Parlodion—Special cellulose nitrate prepared by the DuPont Company.

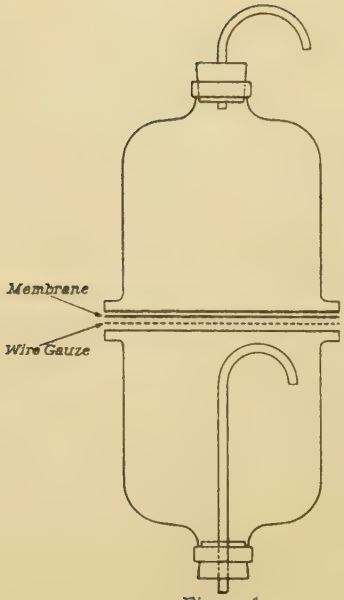

Figure 1.

In Fig. 1, a form of filtration apparatus is shown which was used in these experiments. It consisted of two small bell jars, the upper one serving as the container of the suspension to be filtered and the lower one, fitted with an air outlet tube, as the receiver. Between the two was placed a nichrome gauze which served to support the collodion membrane. The two jars were supported on a ring stand and sealed together by use of a melted rubber-paraffin-beeswax preparation which hardened to a firm joint. Further pressure was applied, to prevent any leakage at the joint, by means of strong rubber bands attached to two wooden arms fitted over the necks of the upper and lower containers.

The filtrations were carried out using gelatinized starch suspensions of varying concentrations, membranes of varying grades with an air pressure of 4 cm. of mercury applied on the surface of the suspension. The filtrate in all cases was

perfectly clear, although the rate of filtration varied somewhat with the permeability of the membrane. The operation was continued until washings gave no further tests for beta amylose with iodine test solution. The solutions thus obtained were analyzed for solid contents by drying a sample on clean, ignited sand at 105° and by polariscope readings taken on the clear filtrate.

TABLE I.

ULTRAFILTRATIONS OF GELATINIZED STARCH "SOLUTIONS"

	I	II	III	IV	V	VI	VII	VIII
Variety of Starch	Wt. of Sample (dry) gms.	Grade of Membrane	Vol. of Filtrate cc.	Wt. Beta gms.	% of Beta	% of Alpha (by diff.)	β Amylose $(\alpha)^{25}$ 546.1 μμ	$(\alpha)^{25}_{D}$ [Calc.]*
Corn	7.44	9.4	100	6.382	85.7	14.3	...	....
Corn	20.00	22.0	330	16.523	82.0	18.0	...	....
Corn	8.00	4.1	117	6.642	83.0	17.0	...	....
Corn	6.07	13.7	143	5.247	86.4	13.6	...	....
Corn	15.00	14.3	470	12.880	85.0	15.0	215	182.4
Corn	10.00	19.0	290	8.360	83.6	16.4	214	181.6
Corn	10.12	15.0	195	8.906	87.9	12.1	214	181.6
				Av.	84.8	15.2		
Rice	10.00	20.0	272	8.146	81.4	18.6	...	....
Rice	9.06	16.0	220	7.346	81.0	19.0	...	....
Rice	10.00	14.0	270	8.284	82.8	17.2	...	....
				Av.	81.7	18.2		
Potato	10.00	13.0	259	9.724	97.2	2.8	214	181.6
Potato	10.00	13.0	256	9.715	97.1	2.9		
				Av.	97.15	2.85		

Attention may be called to the wide range in permeability of the membranes (according to this method of grading)

* $(\alpha)^{25}_{D}$ were calculated from the observed rotations which were determined by the use of a mercury vapor lamp which gives a light of $\gamma = 546.1\,\mu\mu$. The factor 0.8486 as determined by Vosburgh [12] was used. This factor was determined from observed rotations, which were experimentally determined on sucrose and invert sugar solutions using both types of light.

through which beta amylose will pass. (Column II of Table I). The lowest grade membrane used was a grade 2, but owing to the extreme slowness of filtration its use was impractical though some amylose even passed through this membrane.

As shown by averages in column V, there is a striking similarity in the "soluble" or pure carbohydrate content of the first two cereal starches as contrasted with the higher "soluble" content of the tuber starch. This would indicate that if one wished to make starch derivatives from whole starch, potato would be apparently the one to select.

In subsequent experiments where the alpha portion was determined directly and the beta found by difference it will be shown that the ratios as given in Table I are substantially correct.

It is interesting to note the high beta concentrations of these aqueous dialysates (Columns III and IV). These solutions, which are perfectly clear and transparent, can be obtained containing as much as 8% beta amylose. We believe that this is about the highest concentration which has been recorded for a starch solution with these properties.

On long standing, solutions of beta amylose become cloudy and finally deposit material in the form of an insoluble hydrated mass. This has been called reversion or retrogradation and studies on the phenomenon are being carried out in this laboratory at the present time. By not allowing the solution to stand too long retrogradation difficulties were avoided.

Electrodialysis:

Many investigators have mentioned the migration of starch when subjected to the action of the electric current. Samec (loc. cit.) in particular, has studied the conductivity of the amylopectin from potato starch in relation to its phosphorus content. Therefore in the separation of the two amyloses on a larger scale than in the case of ultrafiltration, it was considered possible to use this property.

For this purpose an apparatus, Fig. 2, was assembled, which would allow a cataphoresis of the starch to take place and at the same time adialysis of the small amount of remain-

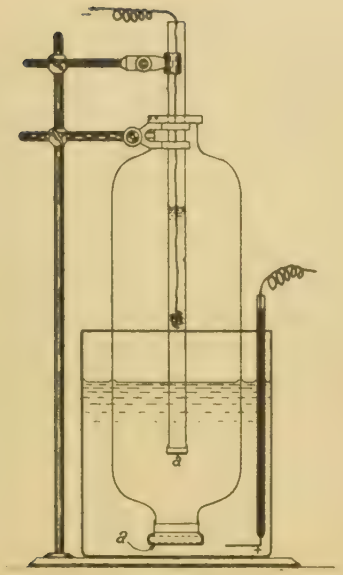

Figure 2.

ing ammonium thiocyanate and any other soluble salts. These served to make the solution conduct the current. A cylinder 65 cm. long and 18 cm. in diameter, which was constricted on either end to an opening of 6 cm. in diameter, was used. On one end an impermeable membrane (a) was prepared by dipping a stretched cheese cloth covering in collodion, drying in air and re-dipping and drying until a membrane resulted which was not permeable to the amyloses but would allow the slow dialysis of inorganic ions. This cylinder was then suspended in a large battery jar which would form one electrode chamber and could be filled to the height of the solution inside, thereby lessening the strain on the membrane when the apparatus was in use. As the second electrode chamber a similar membrane (a) was prepared on one end of a glass tube of 4 cm. in diameter which dipped into the solution to be electrodialyzed. Two platinum electrodes were placed in the elec-

trode chambers and connected with the 110 volt direct current, which thus completed the circuit.

In the operation of this apparatus, samples of the completely gelatinized starch varying from 100-300 gms. were suspended in a sufficient quantity of distilled water to make about an 8% suspension. The starch was well stirred and then placed in the cylinder. The electrode chambers were filled with distilled water to the proper level and a direct current of 110 volts connected so that the positive pole was at the bottom of the apparatus. This was essential since it was found that the alpha amylose migrated to the positive pole* and therefore to take advantage of both effects, migration and gravity, this arrangement was adopted. When the current is applied a flocculation takes place in a very short time and soon a sharp boundary is seen to move in a downward direction. This boundary moves, leaving a clear solution behind, until the flocculated material forms a loose mass at the lower membrane. During the time this separation is taking place the water in the electrode chambers is periodically changed, so that all soluble inorganic material may be completely dialyzed away. After the separation has taken place the upper solution containing the "soluble" or beta amylose may be siphoned off and the total concentration determined by drying a sample in sand at 105° C. The major part of the amylose may be recovered by precipitation with alcohol and washing with absolute alcohol which gives a white, fine powdery product. To continue purification of the residue of alpha amylose in the electrodialysis, distilled water was added again and a second migration allowed to take place, after which the supernatant liquid was drawn off and the concentration determined. This process was con-

* By means of a U tube the effect of an acid or alkaline medium on the migration of the corn starch was studied. Two electrode chambers with impermeable collodion membranes were prepared and placed so as to dip just into the suspension of starch in either arm. Since the effect of gravity would be equal in the two arms, any boundary movement could be attributed to the effect of the applied electric current. In this way the alpha amylose was found to move toward the positive pole whether in an alkaline or acid suspension.

tinued until the "soluble" amylose was entirely washed away from the alpha amylose. The suspension of the latter was frozen[13, 14] and melted, producing a partially dehydrated product which could be filtered readily. After drying with absolute alcohol or in the oven, the amount of alpha amylose by weight was determined.

TABLE II.

EECTRODIALYSIS OF STARCH

	I	II	III	IV	V
Variety of Starch	Wt. of Sample (dry) gms.	Wt. alpha Amylose gms.	Wt. beta Amylose gms. (by diff.)	% alpha Amylose	% beta Amylose
Corn	100.00	11.45	88.55	11.4	88.5
Corn	100.00	14.80	85.20	14.8	85.2
Corn	187.35	25.62	161.73	14.0	86.0
Corn	200.00	27.84	172.16	13.9	86.1
Corn	273.00	37.37	235.63	13.7	86.3
Corn	242.06	37.85	204.22	15.6	84.4
			Av.	14.0	86.0
Rice	100.00	15.91	84.00	15.9	84.1
Rice	85.10	14.50	70.60	17.0	83.0
			Av.	16.5	83.5
Potato	100.00	1.93	98.07	1.9	98.1
Potato	100.00	1.75	98.25	1.7	98.3
			Av.	1.8	98.2

The average results as given in column IV in Table II show the per cent. of alpha amylose as determined by direct weight. In Table I page 11 the results of a direct determination of beta amylose were given. Comparison of these tables shows a good agreement in the amounts of the two amyloses in corn starch:

	Av. % of alpha	Av. % of beta
Ultrafiltration - -	15.4 (by diff.)	84.6
Electrodialysis - -	14.0	86.0 (by diff.)

It can be concluded therefore that the alpha and beta amylose occur in corn starch in a ratio of approximately 15:85. The difference in the methods are due to slight losses and the experimental error.

Polariscope Studies on Fractions of Beta Amylose from Ultra Filtration:

In order to determine the effect of filtration, if any, on the composition of successive portions of the filtrate of beta amylose, polariscope studies were made on filtrations of both gelatinized starch which had been washed with alcohol until free from thiocyanate, and pure beta amylose solutions which had been re-filtered. If the high and relatively constant specific rotatory power and the pure blue color in the iodine test on beta amylose solutions, produced from several independent methods of pretreatment and subsequent separation are an indication that no degradation of the starch has taken place, then our results prove that we have the beta amylose as originally present in the granule.

As shown in Table III, the concentrations of the beta amylose solutions varied from 4 to 7%, the membranes from grade 6 to 16 and the results all fall in a range from 182°-188° which corresponds with the data of Fouard [15] on the rotations of beta amylose solutions in which he gives 181°-190° with an average of 186°. Had there been dextrinization or saccharification during the pretreatment of the starch it is reasonable to expect some fractionation during filtration. In this event the first portions would probably show lower rotatory power and reducing action with Fehling's solution. As will be seen, the rotatory power of successive fractions is sensibly constant. Qualitative tests with Fehling's solution were negative in every instance.

Studies of Methods of Gelatinization:

To determine whether the method of gelatinization employed had any peculiar or special effect on the separation of the amyloses, a 1% suspension of raw corn starch was heat gelatinized in the autoclave under 15 lbs. pressure for one-half

TABLE III.

POLARISCOPE STUDIES ON BETA AMYLOSE FROM CORN STARCH

Material	Wt.	Grade of Membrane	1st fraction % β	1st fraction $[\alpha]^{25}$ 546.1 μμ	1st fraction $[\alpha]^{25}_D$	2nd fraction % β	2nd fraction $[\alpha]^{25}$ 546.1 μμ	2nd fraction $[\alpha]^{25}_D$	3rd fraction % β	3rd fraction $[\alpha]^{25}$ 546.1 μμ	3rd fraction $[\alpha]^{25}_D$	4th fraction % β	4th fraction $[\alpha]^{25}$ 546.1 μμ	4th fraction $[\alpha]^{25}_D$
Gelatinized Starch	8	13.0	7.6	218	184.9	7.5	215	182.4	7.4	219	185.8			
Gelatinized Starch	8	6.7	6.2	222	188.3	6.1	220	186.6	5.1	217	184.9			
Gelatinized Starch	8	6.8	7.1	218	184.9	6.8	221	187.5	6.1	221	187.5	5.3	220	186.7
Gelatinized Starch	6	12.7	5.3	219	185.8	5.3	222	183.3	5.2	218	184.9	4.5	221	187.5
Redissolved Beta	–	16.0	6.06	222	188.3	6.2	220	186.6	5.3	219	185.8	4.8	220	186.7

hour. At the end of this time it was found to be completely gelatinized as observed under the microscope. This solution was then subjected to electrodialysis in a small apparatus and after a few hours time it was found to have a distinct boundary movement as with the thiocyanate treated sample. However, the layer which separated was not a flocculent solid but rather a gelatinous mass, which made the separation incomplete and therefore impractical for our purpose.

Likewise when such a sample was ultrafiltered it was found that the hydrated mass so clogged the pores of the collodion filter that even in several weeks only a few cc. could pass through. It was interesting to note however that the beta amylose solutions obtained in either case from corn starch gave a distinct blue with iodine, hydrolyzed to a clear solution (no fat), and gave an $(\alpha)_D^{25}$ of 186.7°. This seems to indicate that the beta amyloses obtained by either method of gelatinization are the same.

Samples of the alcohol-hydrogen chloride purified starch, just neutralized with sodium carbonate, were gelatinized by heating in the autoclave at 15 lbs. pressure for one-half hour. When this material was ultrafiltered a clear filtrate was obtained and the percentage of beta amylose corresponded with those obtained from the separations on the thiocyanate gelatinized starch, as shown in Table IV.

TABLE IV.

ULTRAFILTRATION OF ALCOHOL-HCL TREATED CORN STARCH NEUTRALIZED AND HEAT GELATINIZED.

Sample gms.	Grade of Membrane	Vol. of Filtrate cc.	Wt. Beta gms.	% Beta Amylose
24.7	13	500	21.1	85.5
10.0	13	297	8.7	86.7
10.0	14	288	8.7	87.3

The above data indicates that the same results are obtained by pressure gelatinization as by thiocyanate gelatinization but because of ease in handling and higher concentrations obtained the latter method is by far the better.

Properties of the Amyloses:

The two amyloses differ greatly in their physical and chemical properties. Alpha amylose is insoluble in water and when dried either by alcoholic treatment or in the air forms a light brown hard material whereas the beta amylose is soluble in water over a wide range, 1% to 8% by weight and may be precipitated with alcohol giving a fine, pure white powder.

The beta amylose solution serves as a good indicator in iodimetry, giving a clear deep blue color, whereas the alpha amylose gives a reddish violet or purplish color when in suspension.

In the electrodialysis the alpha amylose moved when a potential difference was applied while the beta amylose did not appear to move. The beta amylose when burned left no weighable ash. This would indicate the absence of any inorganic constituents, while the alpha leaves a slight residue on ignition.

When subjected to hydrolysis using 10% hydrochloric acid the alpha amylose is attacked slowly but when completely disintegrated leaves a flocculent residue much the same as that produced by the original starch when it is hydrolyzed. In contrast, the beta amylose hydrolyzes readily and gives a clear colorless solution with the characteristic reducing properties of a sugar. This condition upon hydrolysis may serve well as a criterion of purity of the beta amylose along with its characteristic rotatory power of 186°, its color with iodine, its solubility, its non-reducing properties before hydrolysis, and the absence of ash upon burning.

Distribution of Non-Carbohydrate Constituents:

The flocculent residue from the hydrolysis of alpha amylose upon extraction with ether yielded a fatty residue, a property which has been shown to be characteristic of whole corn starch (loc. cit.). In order to make sure that the fatty material was not absorbed as free fat, a sample of alpha amylose was extracted in a Soxhlet for eighteen hours using carbon tetrachloride as the solvent. By this treatment 17.353 gms. of alpha amylose yielded 0.066 gms. extractable residue. Upon hydro-

lysis this same sample yielded 0.2042 gms. or 1.18% of fatty material which would indicate that the fatty material was not liberated until hydrolysis had taken place. Samples of this material were found to have an iodine number of 90.6 and 91.2 which is the same as those of the fatty acids which may be extracted from commercial refinery mud. (Taylor and Nelson, loc. cit.)

However, during each successive treatment of the starch preparatory to the actual separation of the alpha amylose, a small amount of fat was unavoidably lost, so that the actual percentages decreased slightly at each stage.

TABLE V.

FAT STUDIES ON CORN STARCH

Material	% Fat in Raw Starch	% Extraneous Fat	% Fat on HCl treated Starch	% Fat on NH_4CNS treated Starch	% Fat in Alpha amylose
Old Starch	0.82	0.17	0.47	0.297	1.6
Old Starch	0.79	0.12	0.47	0.297	1.5
New Starch	0.61	0.04	0.55	0.359	2.3
New Starch	0.59	0.03	0.54	0.350	2.3

The first column of Table V shows the percentages of fatty material in the raw starch from both old and new preparations. The second series show the small amount of extraneous fat as determined directly on a sample of untreated starch. This amount would be expected to be lost during the alcohol-hydrogen chloride treatment. Then a small amount is lost during gelatinization but the alpha amylose is found to be much higher in fat which indicates that the combined fat stays in the alpha amylose during separations, which is corroborated by the lack of any fatty material in the beta amylose.

Further the amount of fat carried by the alpha amylose approximates the total carried by the thiocyanate treated material of which the alpha is a part.

TABLE VI.

FAT RECOVERY STUDIES

Material	Wt. of NH_4CNS treated sample gms.	% Fat	Wt. Alpha Amylose	% Fat	% Fat Recovery
Old Corn Starch	100	0.30	11.5	1.6	60
Old Corn Starch	100	0.30	14.8	1.5	73
New Corn Starch	187	0.36	25.6	2.3	90
New Corn Starch	200	0.36	27.8	2.3	91
New Corn Starch	273	0.36	37.4	2.3	90
Rice Starch	100	0.54	15.9	2.7	82
Rice Starch	85	0.40	14.5	2.1	86

For instance, as shown in the corn starch sample of Table VI it was possible to recover from 80%-90% of the fatty material after the separation of the alpha amylose. The rice starch bears a slightly higher percentage of fat and of alpha amylose and in this case also the recovery averaged about 85%.

In the case of the potato starch the percentage of ether extractable material in the raw product was found to be only 0.02%. However, the phosphorus content (Thomas[16]) of potato starch is quite appreciable so it was of interest to see if this separation would likewise separate the phosphorus bearing constituent entirely in the alpha amylose. This was found not to be the case, for the percentage of phosphorus in raw starch and that in the beta amylose were approximately the same, showing no separation had taken place. The phosphorus as determined by the alkaline fusion method described by Sherman[17] was found to be 0.087% and 0.090% in the raw starch, 0.086% in the beta amylose and 0.049% in the alpha amylose. This result might be anticipated since in the work on phosphorus in potato starch of Nelson and Northrop[18], a compound bearing the phosphorus in greater percentage than the original starch was not produced until the starch had been broken down partially by acid hydrolysis and no precipitate with alcohol could be obtained.

SUMMARY

1. The alpha and beta amyloses may be completely separated by electrophoresis.

2. The beta amylose may be ultrafiltered through collodion membranes of proper permeability and thus separated from the alpha amylose.

3. The results from these two methods as applied to corn, potato and rice starches were found to be sensibly the same for each individual starch.

4. The fatty material in corn and rice starches was found entirely in the alpha amylose.

5. The phosphorus in potato starch was found to be distributed in both the amyloses.

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